SOME ISSUES RELATED TO THE ACCURACY AND INTEPRETATION OF PLATELET VIABILITY MEASUREMENTS BY RADIO LABELING STUDIES

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Results of data presented were obtained from studies conducted at
American Red Cross Research Department, Mid Atlantic Region

ISSUES RELATED TO THE ACCURACY AND INTERPRETATION OF VIABILITY MEASUREMENTS

Donor Variability in % Recovery

- Inaccurate Estimation of Blood Volume
- Splenic Uptake
- Periodic variability

Labeling Method/Procedure

- Representative population
- Platelet Damage/Aggregates
- Isotope binding characteristics
- Contaminating cells

Data processing and interpretation

- Data points to Include
- Mathematical Models
  - Fitness of data
  - Robust and meaningful parameters

ISSUES RELATED TO THE ACCURACY AND INTERPRETATION OF VIABILITY MEASUREMENTS

Variability in % Recovery related to the Donor

- Inaccurate Estimation of Blood Volume by body surface area

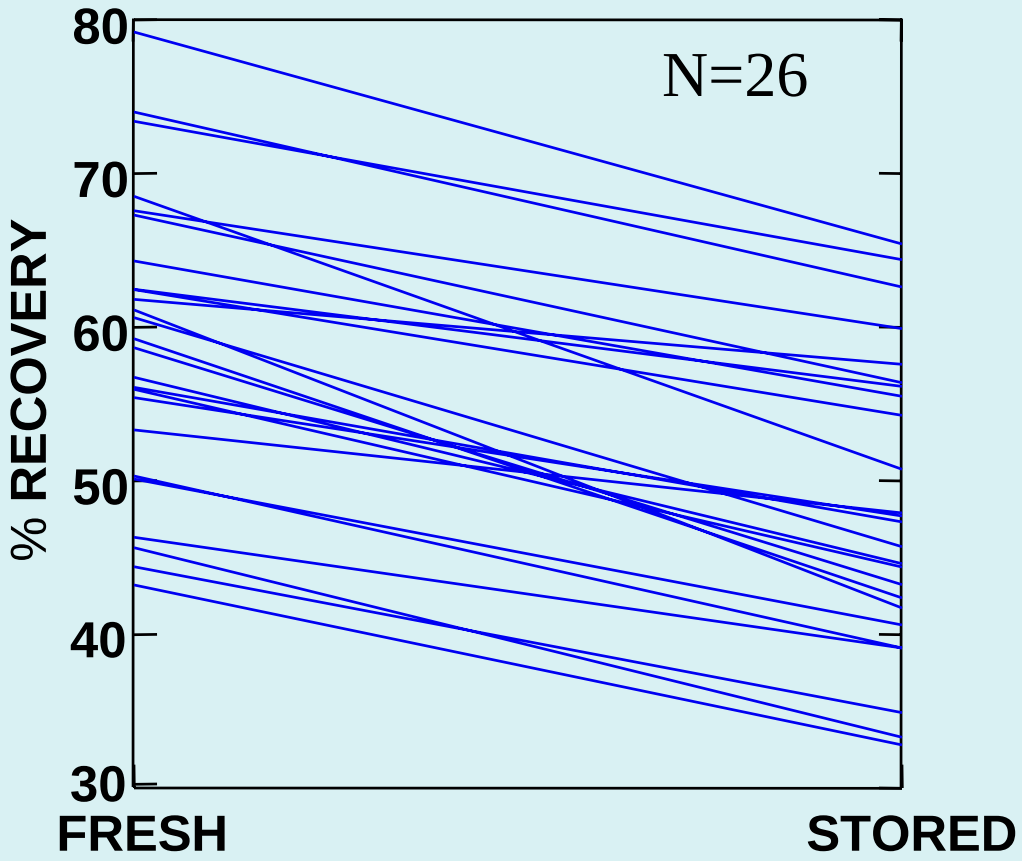
Labeling Method

- Representative platelet population from the product

Data processing and interpretation

- Data points to include
- Mathematical Models
  - Fitness to raw data
  - Robust and meaningful parameters
  - Models comparing test to fresh platelets

VARIABILITY IN % RECOVERY
FRESH VS. 5 DAY STORED PC



VARIABILITY IN % RECOVERY

SOURCE OF VARIABILITY WITH 5 DAY % RECOVERY BY REGRESSION ANALYSIS:

SUM OF SQUARES:

	% OF TOTAL
Regression (fresh)	79 %
Residual (storage lesion)	21 %

(r squared = 0.79)

79 % of the variability is related to the recovery of fresh platelets from the donor and only 20 % is related to product platelet viability during 5 days storage

VARIABILITY IN % RECOVERY

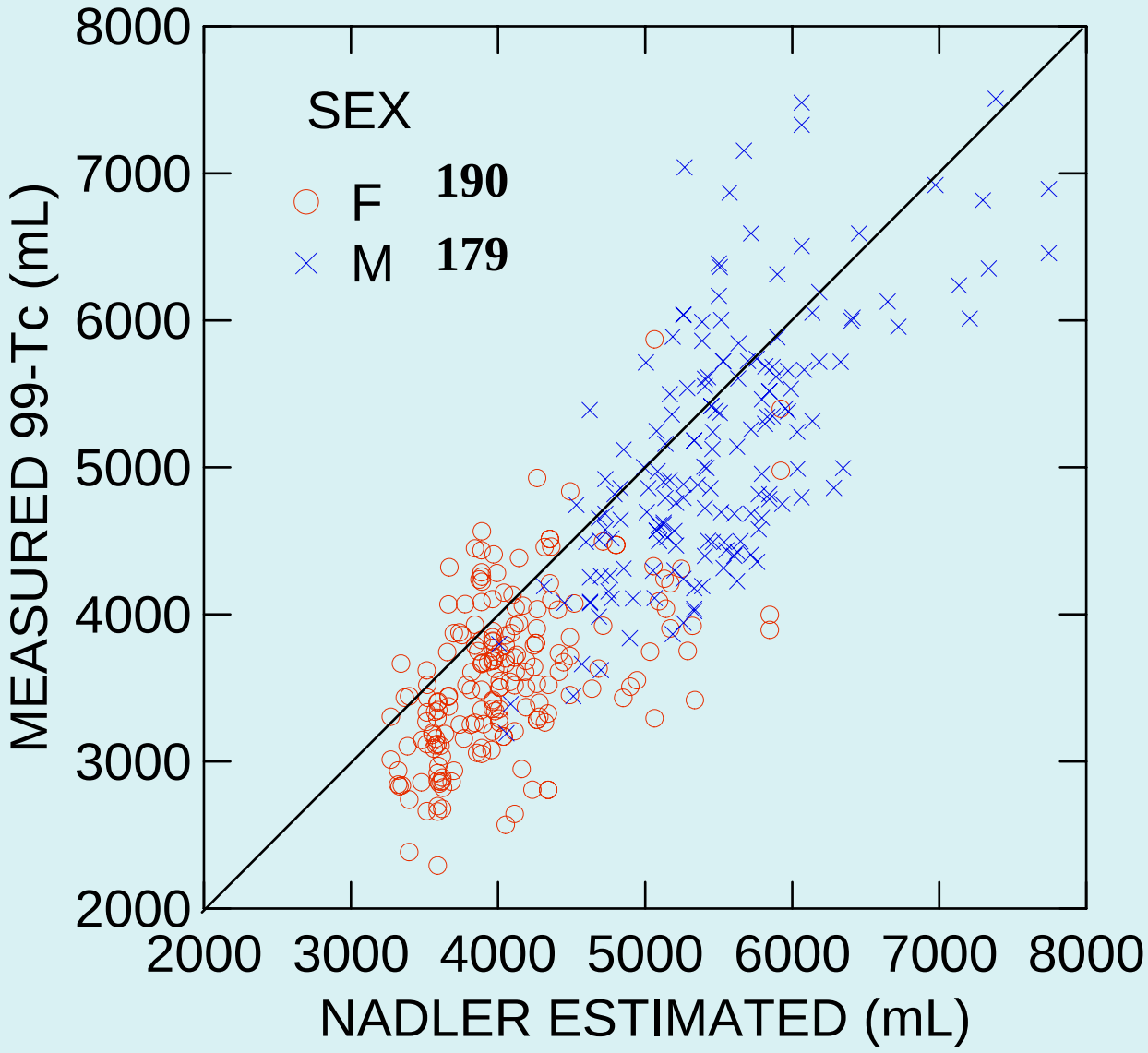
Importance of Accurate Estimation of Blood Volume:

% RECOVERY =

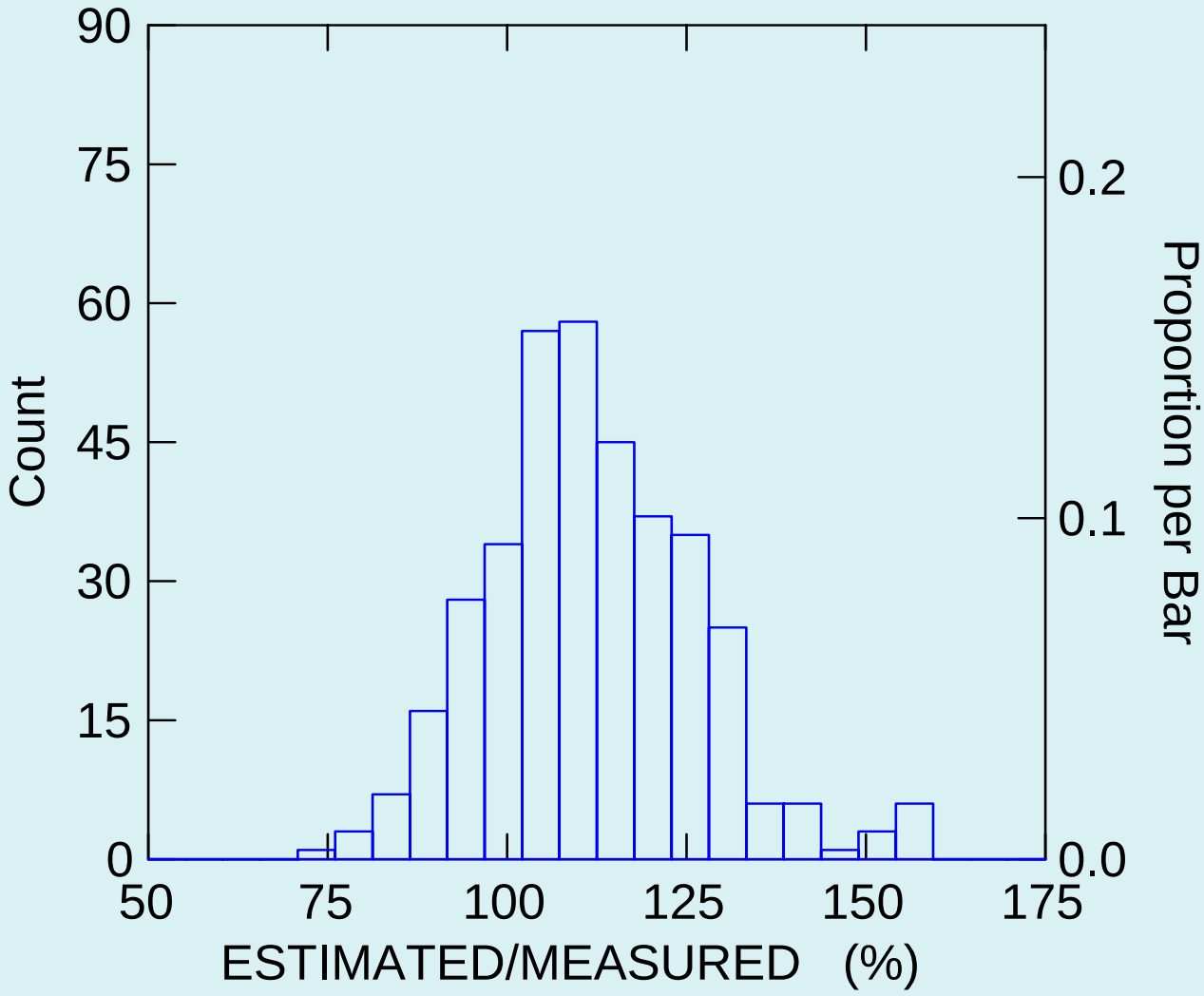
$$\frac{\text{Radioactivity per mL Blood} * \text{Blood Volume (mL)} * 100\ \%}{\text{Radioactivity of the Injectate}}$$

Estimation of blood volume is commonly done by formulas (NADLER) for body surface area

VARIABILITY IN % RECOVERY:
Donor's Blood Volume: Estimated vs. Measured



VARIABILITY IN % RECOVERY:
Blood Volume: Estimated vs. Measured



VARIABILITY IN % RECOVERY

A major source in variability in % recovery of a 5 day stored product is related to inaccurate estimation of the donor's blood volume and relatively little to the viability of the platelet product after storage :

- **The determined % Recovery is not, by itself, an accurate measurement of the platelet viability of a 5 day standard product**

- **Paired Studies (test vs. control products from the same donor) is thus preferable for determination of a potential change in platelet viability of a test as compared to a control product**

LABELING A REPRESENTATIVE PLATELET POPULATION OF THE TEST PRODUCT

Major assumption in radiolabeling studies:

Determination of platelet viability by radiolabeling is based on the assumption that platelets in the product population are uniformely labeled:

(that the amount of radioactivity per platelet is the same for all the platelets)

Thus, after infusion, a % decrease in radioactivity represents certain % loss of the number of injected platelets from circulation.

LABELING A REPRESENTATIVE PLATELET POPULATION OF THE TEST PRODUCT

Assuming two populations of platelets in a product consisting of 50% viable and 50% damaged and nonviable platelets.

1)*The uptake of isotope for the viable is 80% and for the non viable subpopulation 20 % of total radioactivity.*

After infusion the total population of the non viable is removed representing a loss of 50 % of the total platelet population. However, the loss of % radioactivity (% recovery) is only 20 %.

2) *The non viable population are platelets that are lost during the labeling procedure.*

Only the viable platelets are infused - no loss in % radioactivity (% recovery)

LABELING A REPRESENTATIVE PLATELET POPULATION OF THE TEST PRODUCT

Do platelet subpopulations from freshly collected whole blood differ in terms of viability?

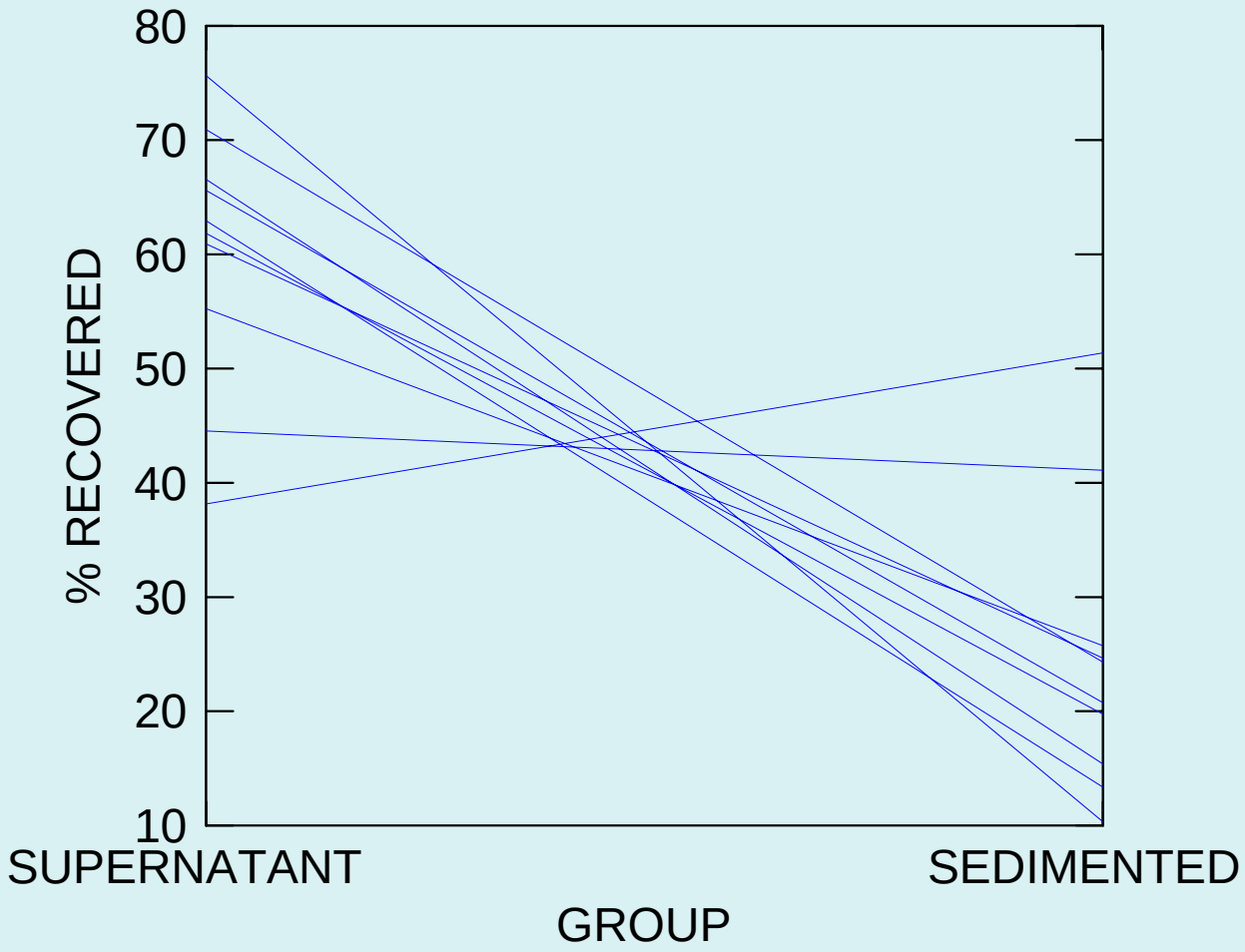
Study Design

PRP (supernatant) platelet subpopulation was prepared by standard centrifugation using random donor WB units (n=8).

The remaining buffy coat (sedimented) platelet subpopulation were obtained by additional processing.

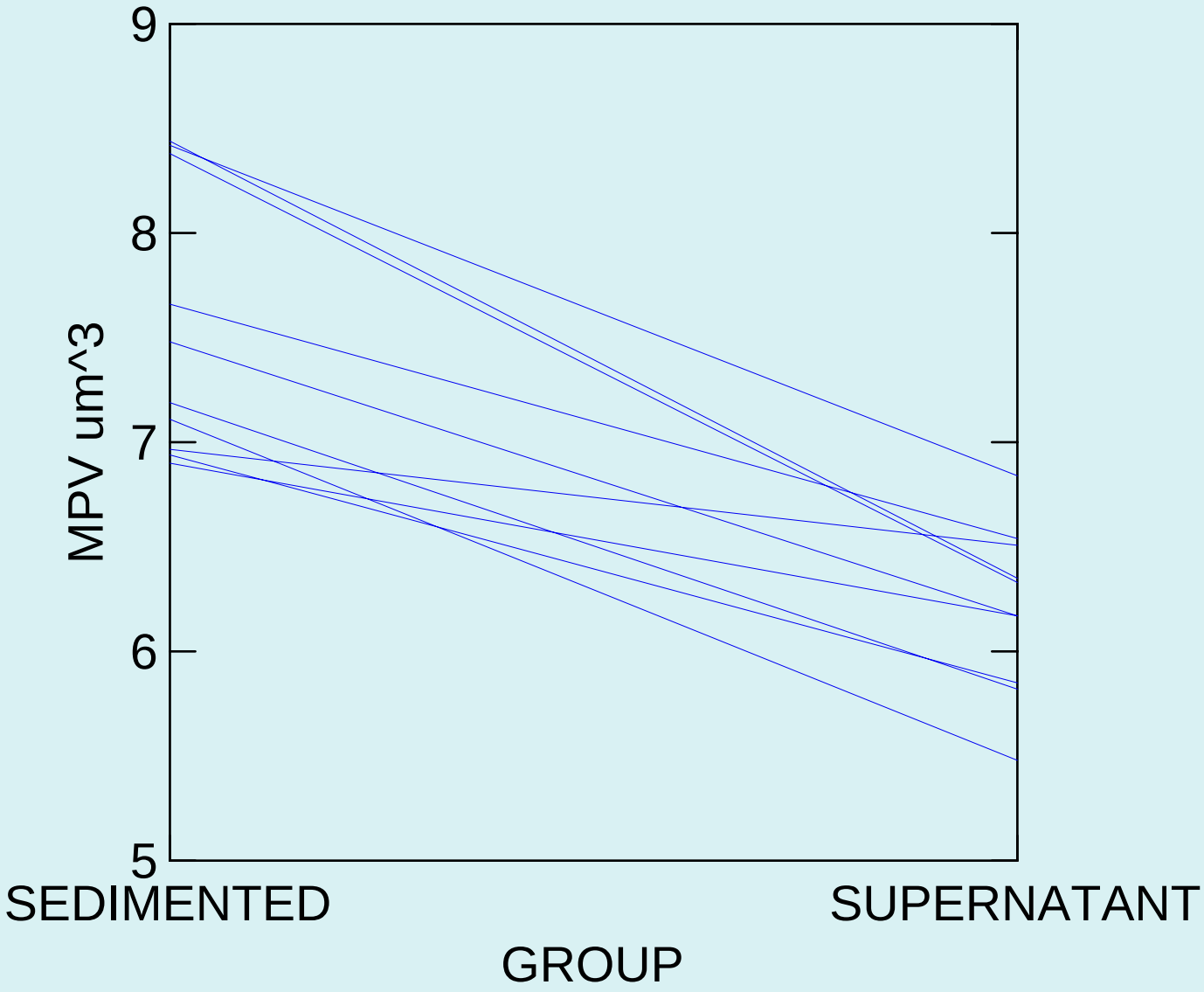
In vivo studies were conducted to determine viability of these two platelet subpopulations using simultaneous labeling and infusion with 111-In and 51-Cr

LABELING OF PLATELET SUBPOPULATIONS:
PLATELET COUNT RECOVERED FROM WHOLE BLOOD



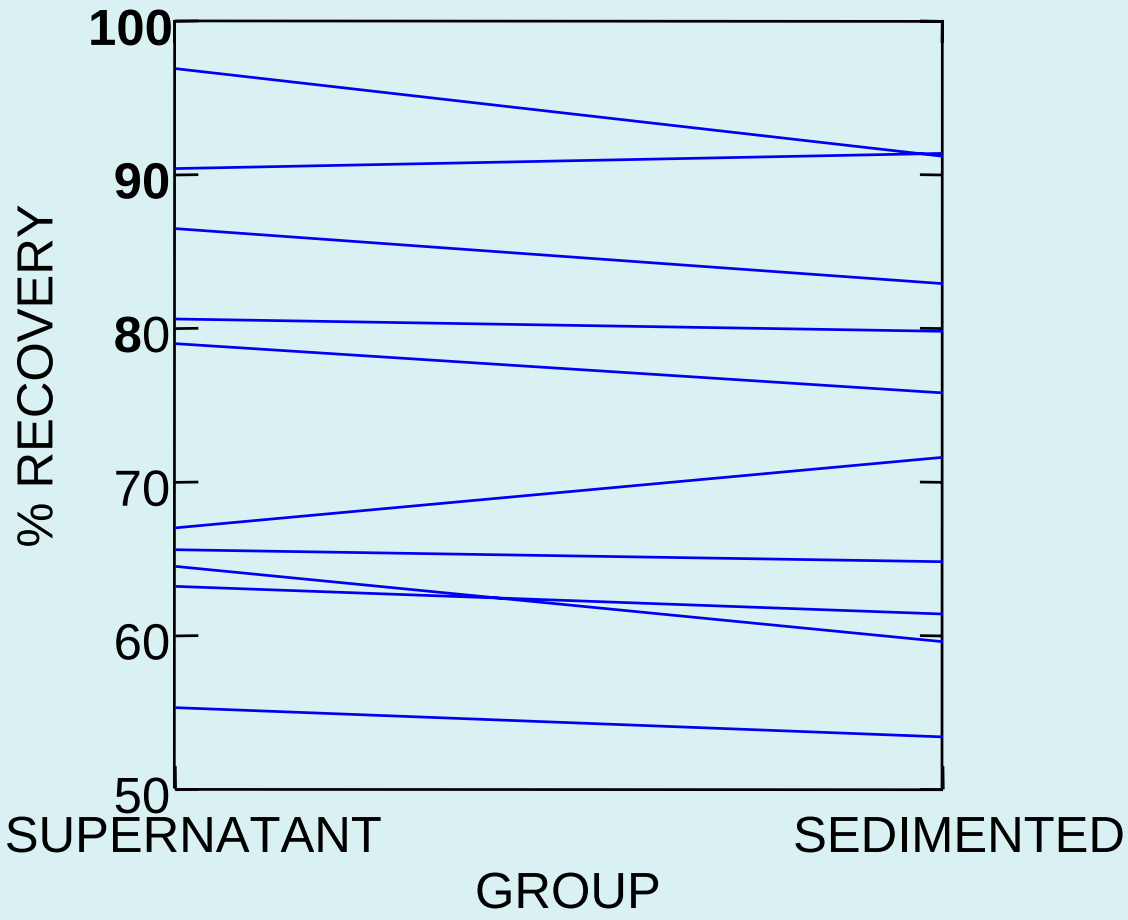
LABELING OF PLATELET SUBPOPOULATIONS:

PLATELET SIZE -MPV



LABELING OF PLATELET SUBPOPULATIONS :
IN VIVO VIABILITY - % RECOVERY

Mean SEDIMENTED = 73.2 (13.3) %
Mean SUPERNATANT = 74.9 (13.7) %

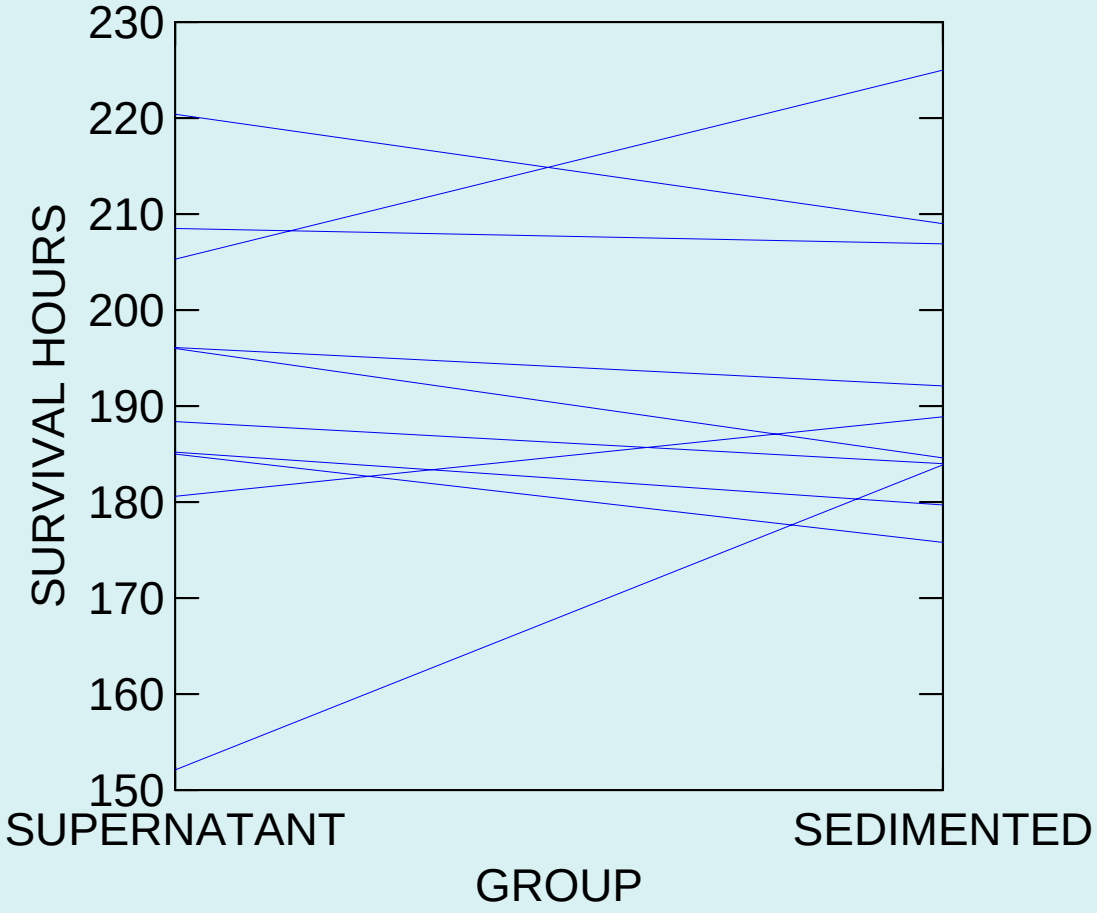


LABELING OF PLATELET SUBPOPULATIONS:

SURVIVAL (NUMERIAL EXPECTED LIFESPAN)

Mean SEDIMENTED = 193 (17) HRS

Mean SUPERNATANT = 192 (19) HRS



LABELING OF PLATELET SUB POPULATIONS

Labeling of platelet subpopulations from freshly collected whole blood

Conclusions:

Using freshly collected blood two platelet subpopulations separated by size showed no statistically significant difference in % recovery and survival.

No statistically significant difference between results obtained using 111-In vs. 51-Cr .

DATA PROCESSING AND INTERPRETATION

Mathematical modeling of the raw data :

Objective

Reduce the data to a few accurate and meaningful parameters that be used to evaluate platelet viability of a product

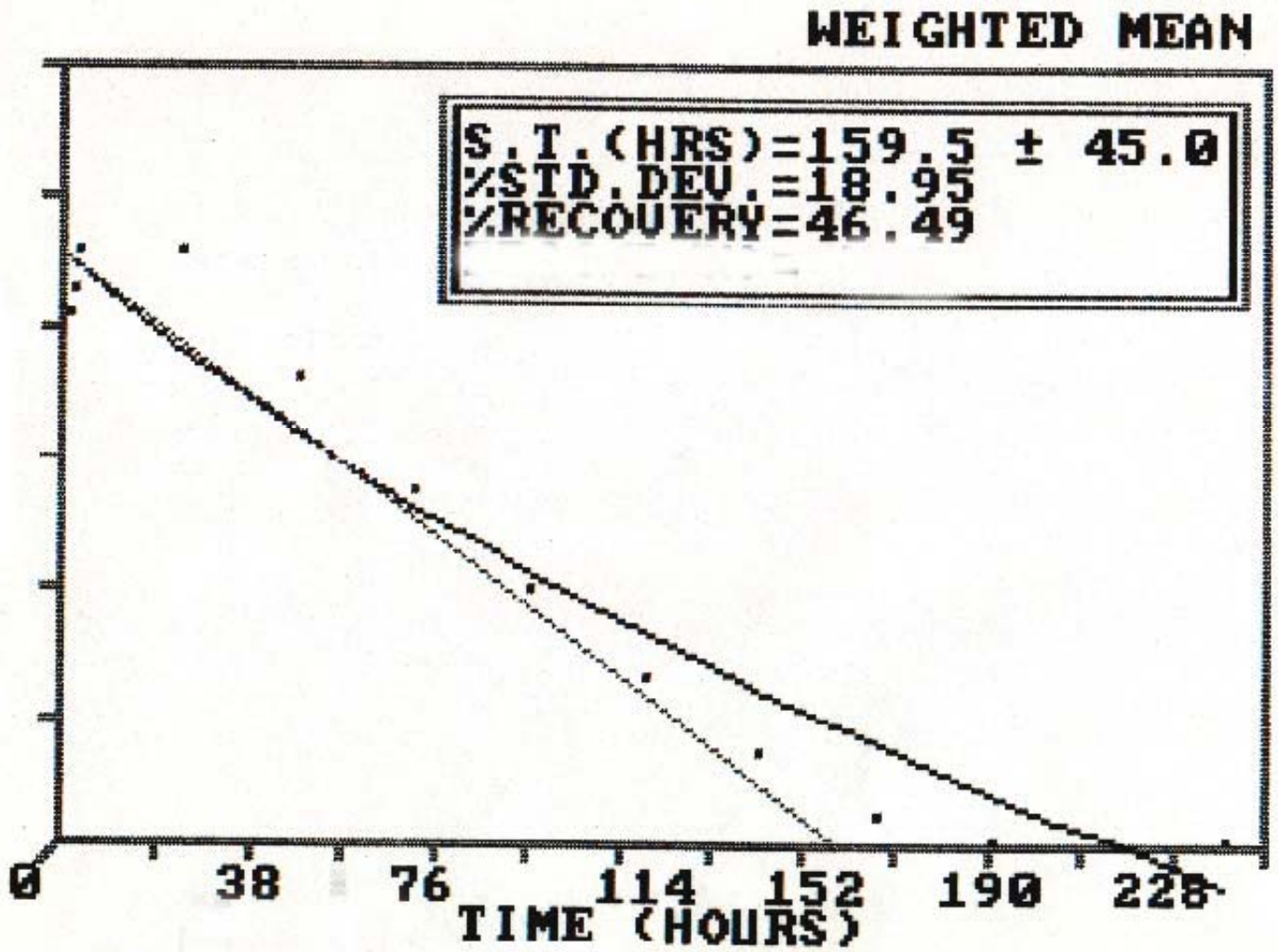
Data points to include?

Method:

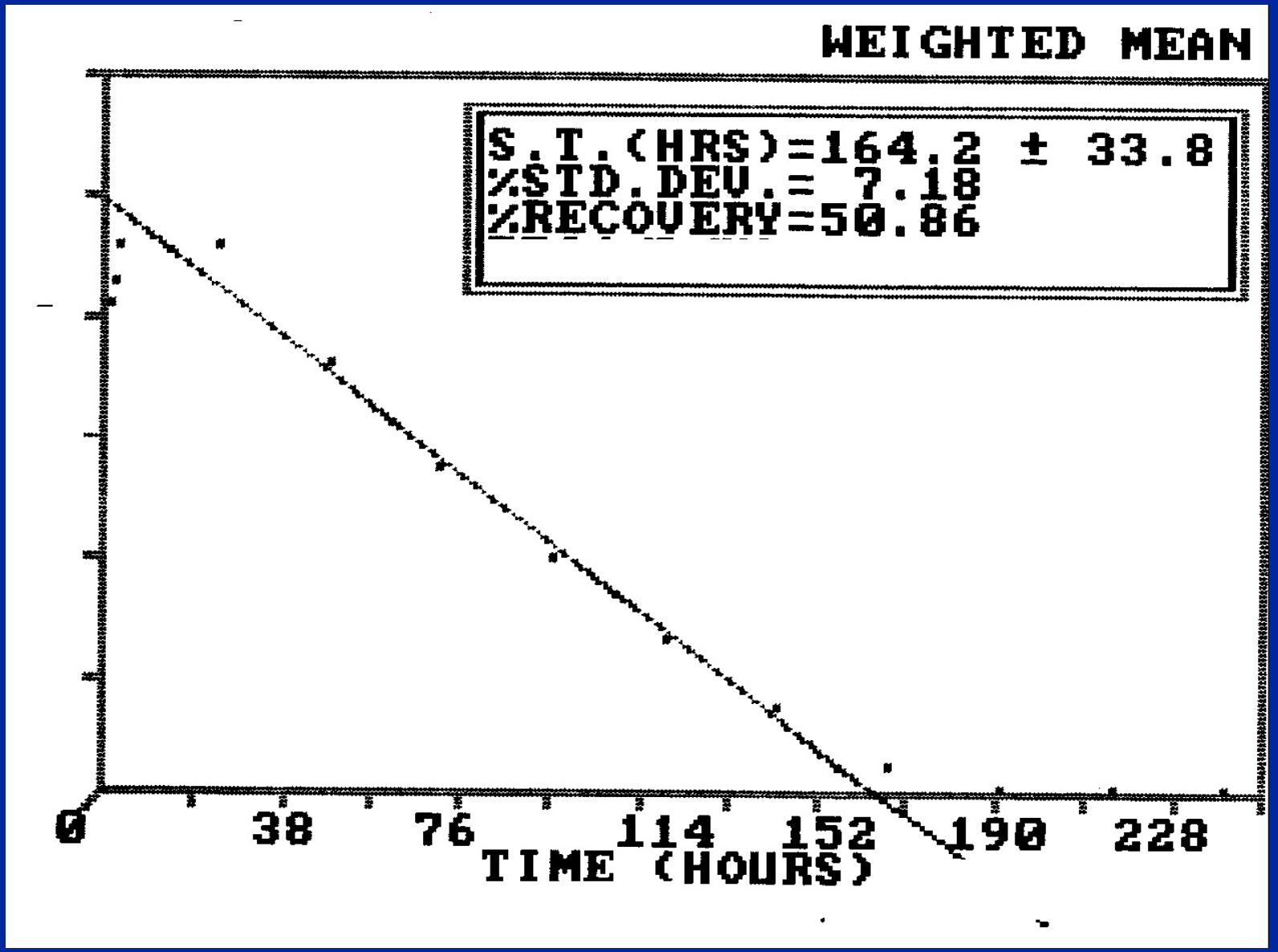
Least Sum Of Squares:

Minimize residual sum of squares = (Observed values – Model Predictions)^2 by iterative methods

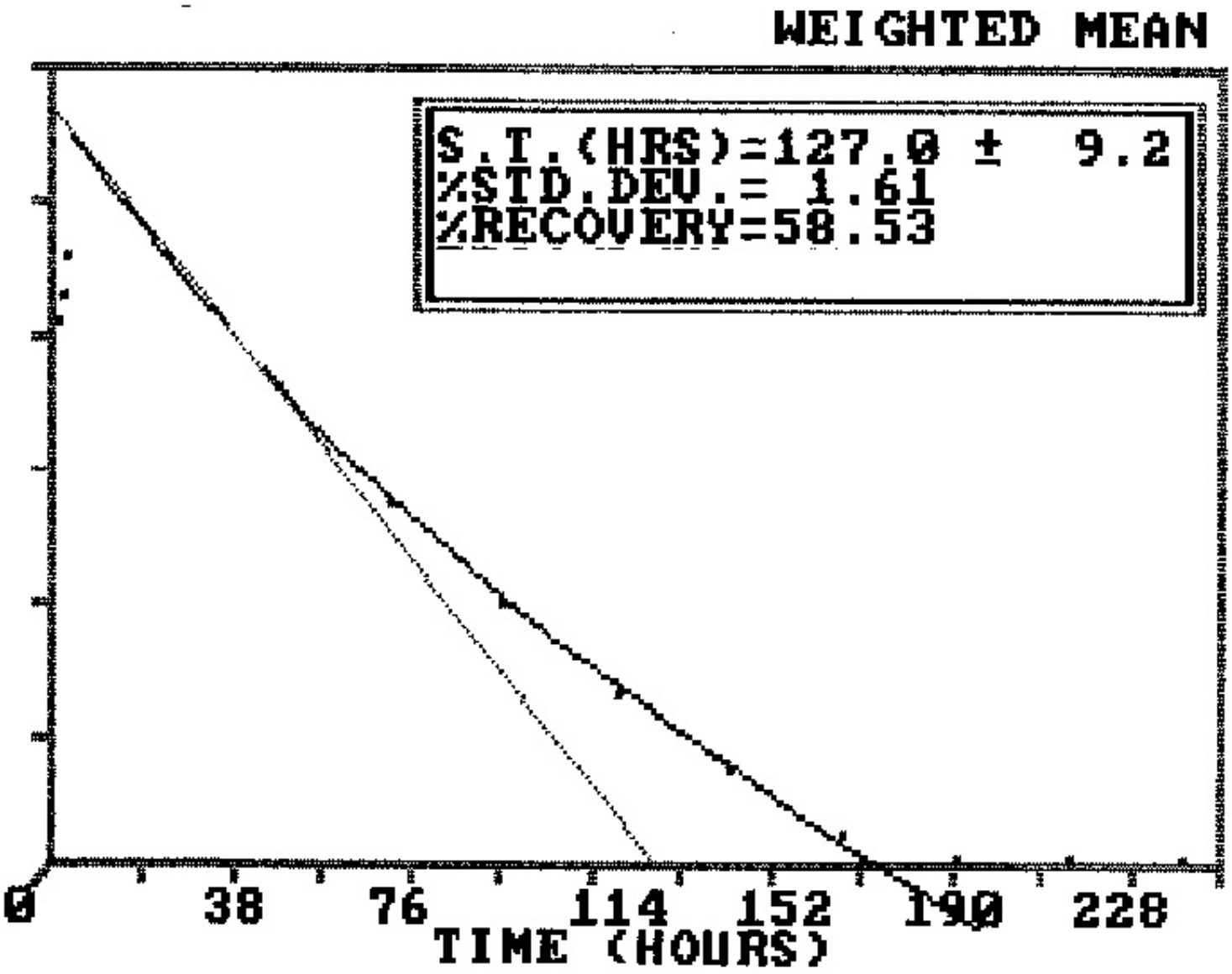
NUMERICAL EXPECTED LIFESPAN – All Data Points



NUMERICAL EXPECTED LIFESPAN – 3 h and daily Data Points



NUMERICAL EXPECTED LIFESPAN – 24 h and daily Data Points



MATHEMATICAL MODELING

Models used in platelet survivals:

- **Linear**
- **Exponential**
- **Multiple Hit (gamma function)**
- **Weighted Mean**
- **Meuleman**
- **Dornhorst**

Requirement:

- **Must be able to fit a wide variety of typical survival curves for platelets stored/processed under various of conditions**
- **The goodness of fit is determined by the residual sum of squares**

MEASUREMENTS OF PLATELET SURVIVAL

Numerical Expected Lifespan :

Intercept of the initial tangent of the survival curve with the x-axis (time)

Mean Residual Lifespan:

Area below the survival curve/%Recovery

T half:

Time after infusion at 50% of initial radioactivity

NUMERICAL EXPECTED LIFESPAN

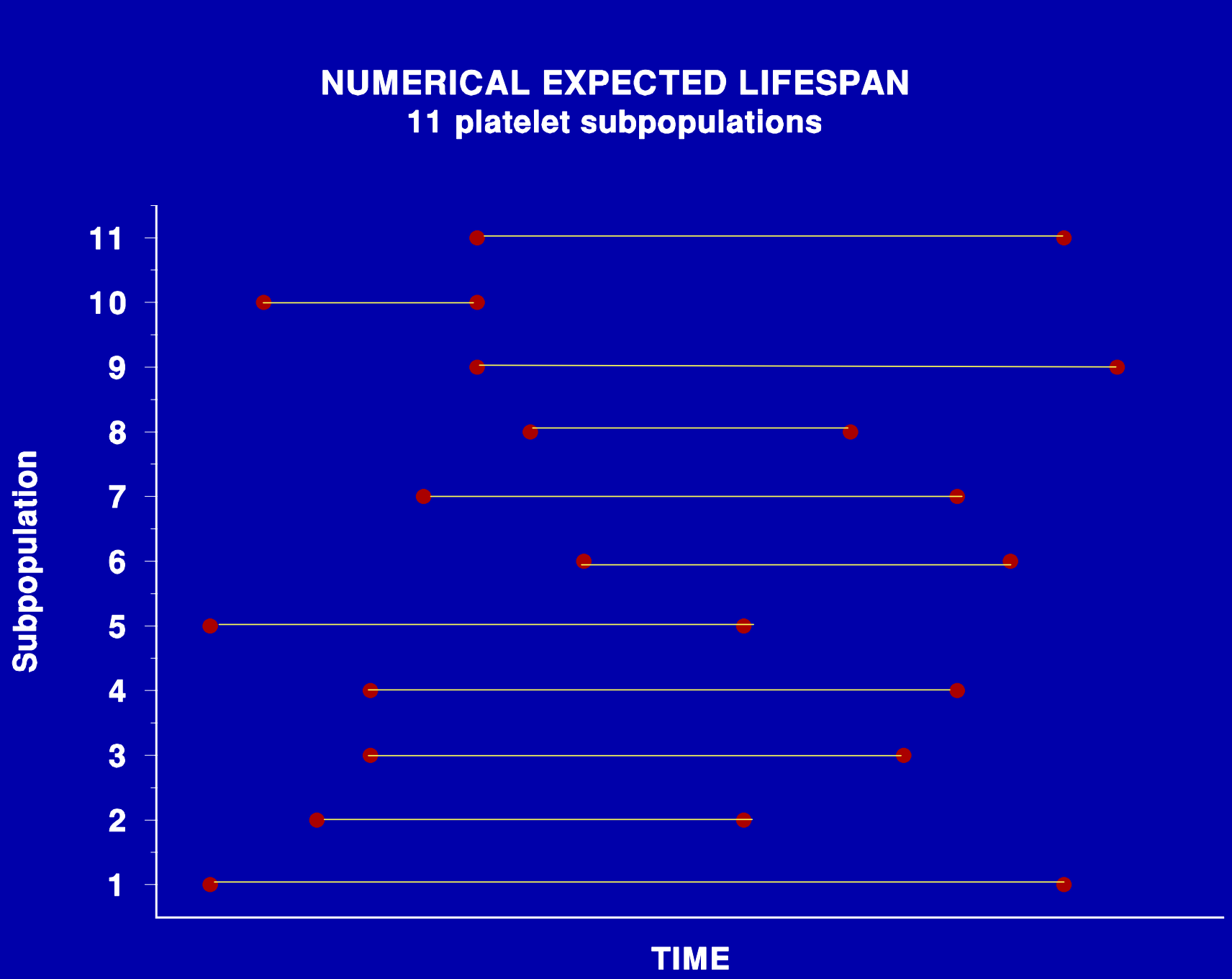
Definition:

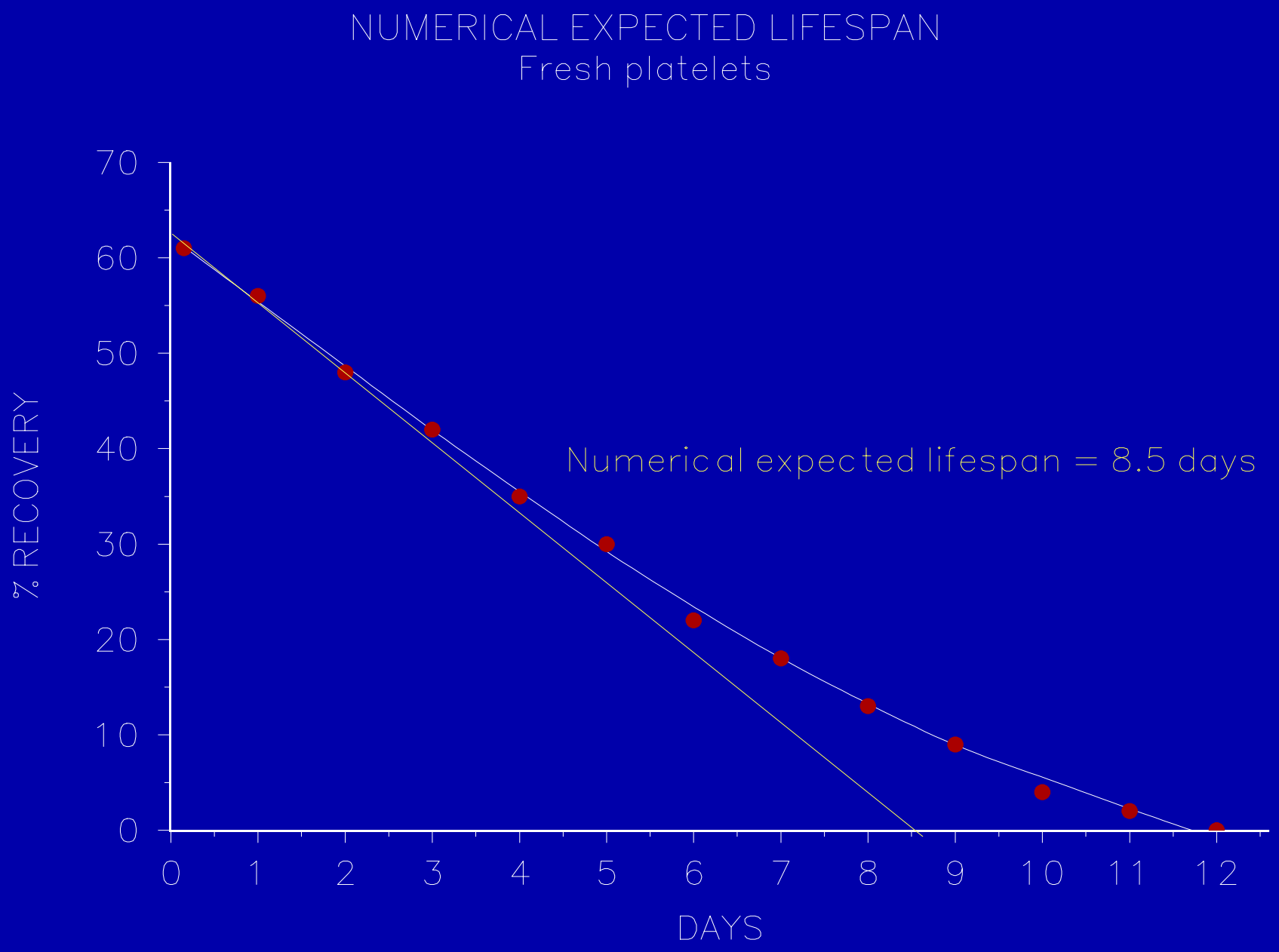
- **Birth cohort lifespan of platelets newly released from the bone marrow**

Used in estimation of platelet survivals in thrombocytopenic patients to determine:

- **Platelet turnover rates**
- **Events in the circulation system (senescence vs. random destruction**

Meaningful in estimation of the survival of platelets in a product?


NUMERICAL EXPECTED LIFESPAN
11 platelet subpopulations
Subpopulation
11
10
9
8
7
6
5
4
3
2
1
TIME

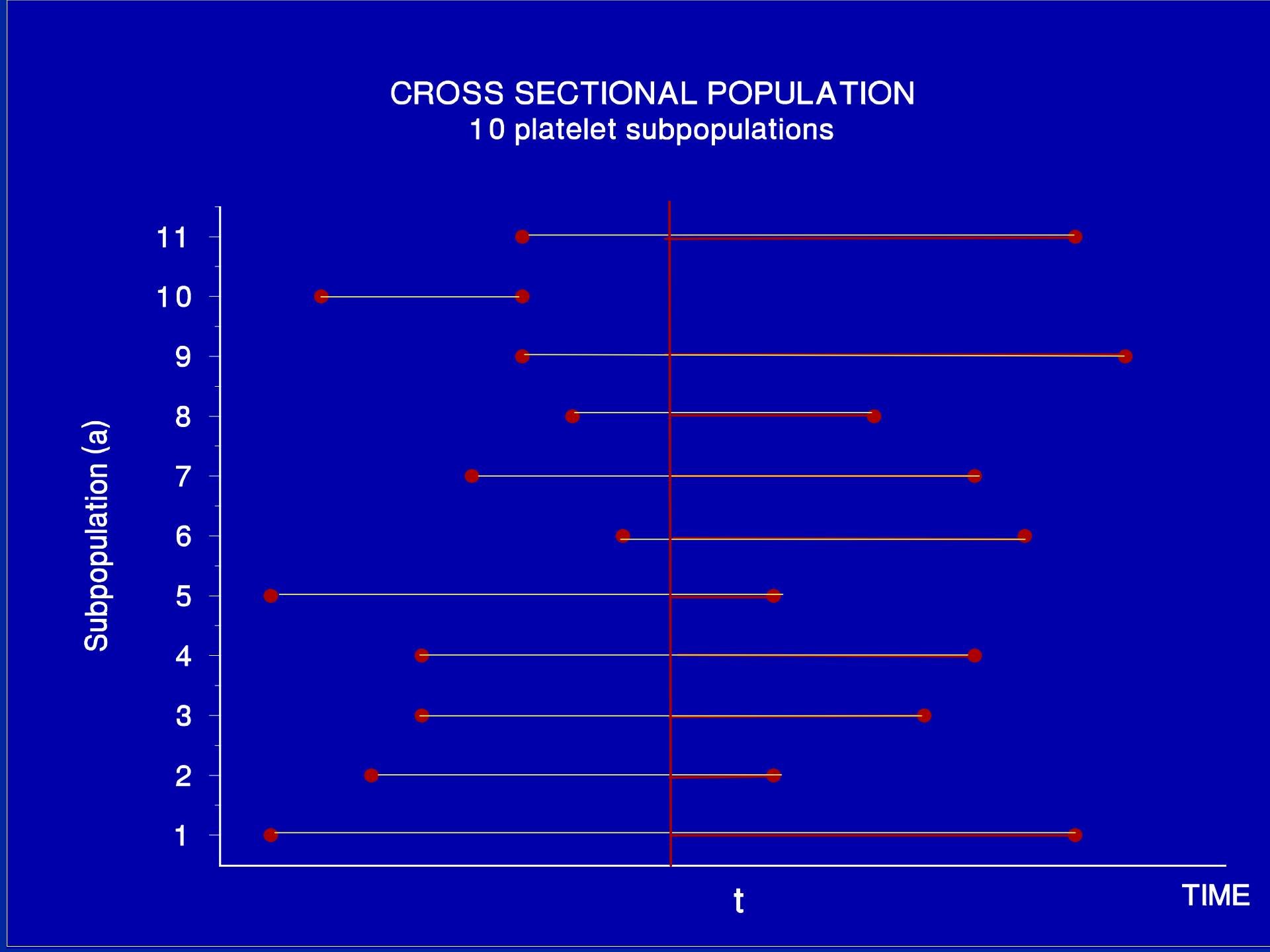

NUMERICAL EXPECTED LIFESPAN
Fresh platelets
% RECOVERY
70
60
50
40
30
20
10
0
0
1
2
3
4
5
6
7
8
9
10
11
12
DAYS
Numerical expected lifespan = 8.5 days

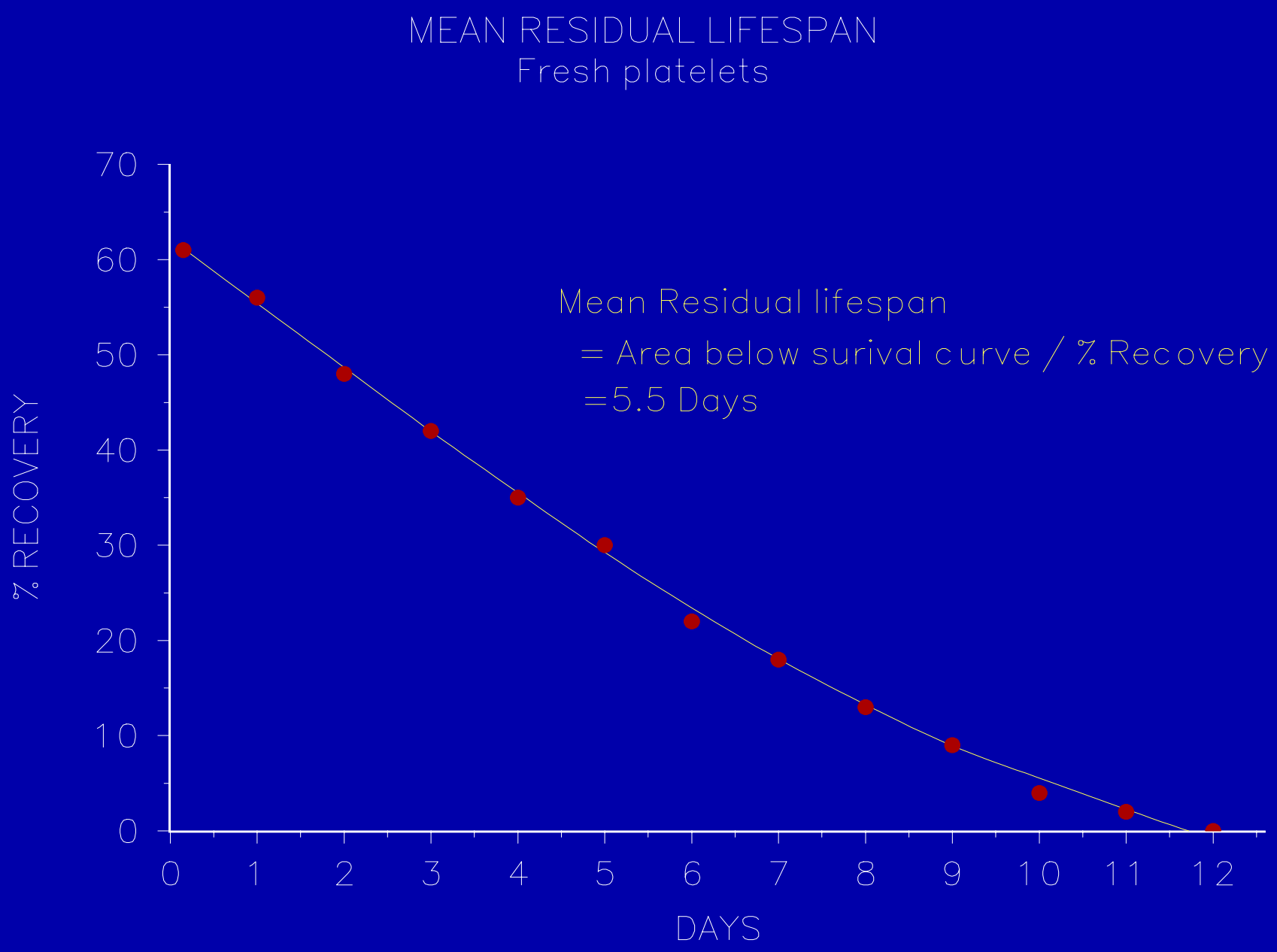
RESIDUAL LIFESPAN

Definition:

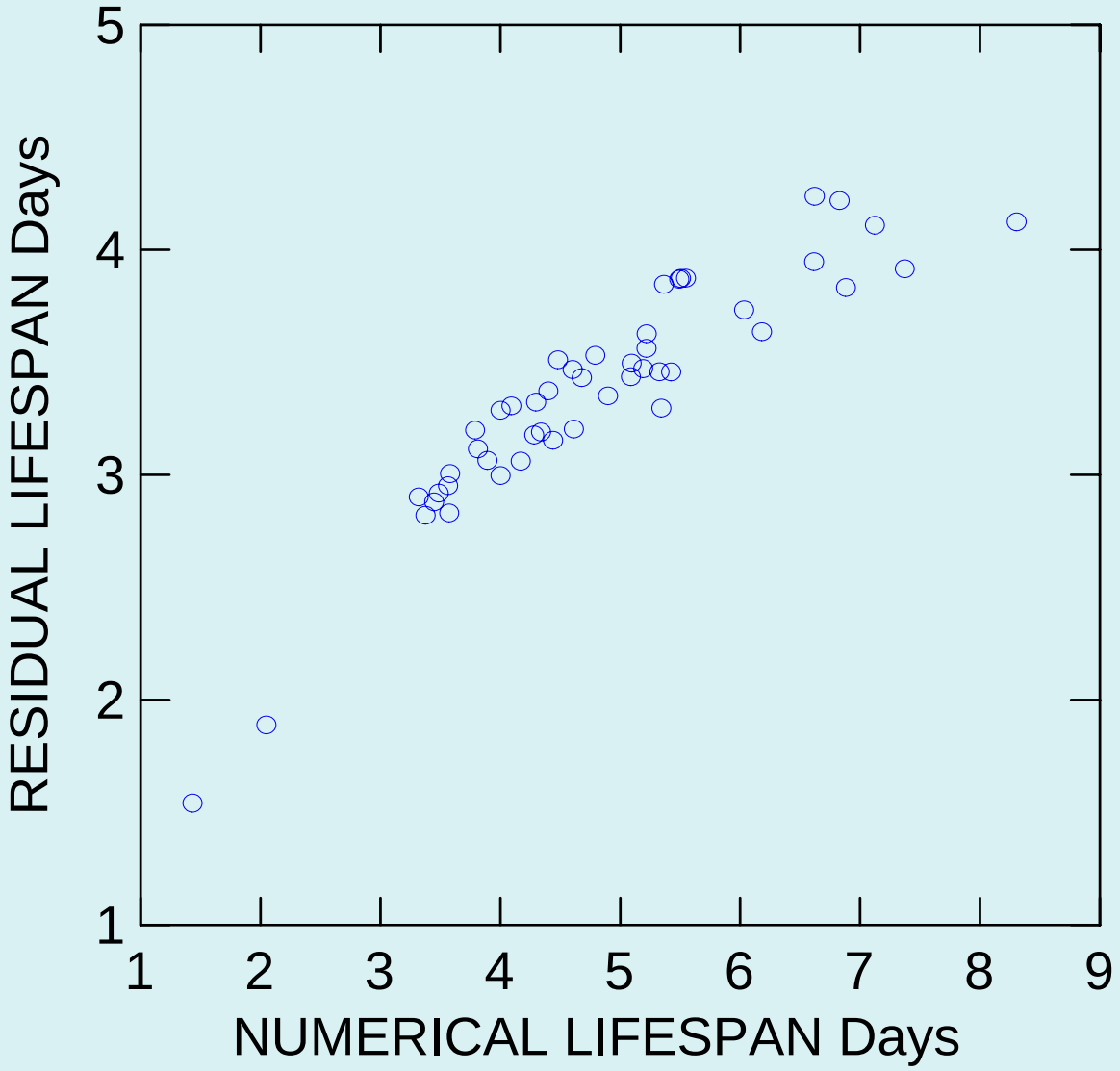
- **Mean residual lifespan in circulation of the labeled and infused platelet population (cross-sectional or sample population)**

- **More robust and meaningful in determination of the viability of a platelet product?**


CROSS SECTIONAL POPULATION
10 platelet subpopulations
Subpopulation (a)
11
10
9
8
7
6
5
4
3
2
1
t
TIME

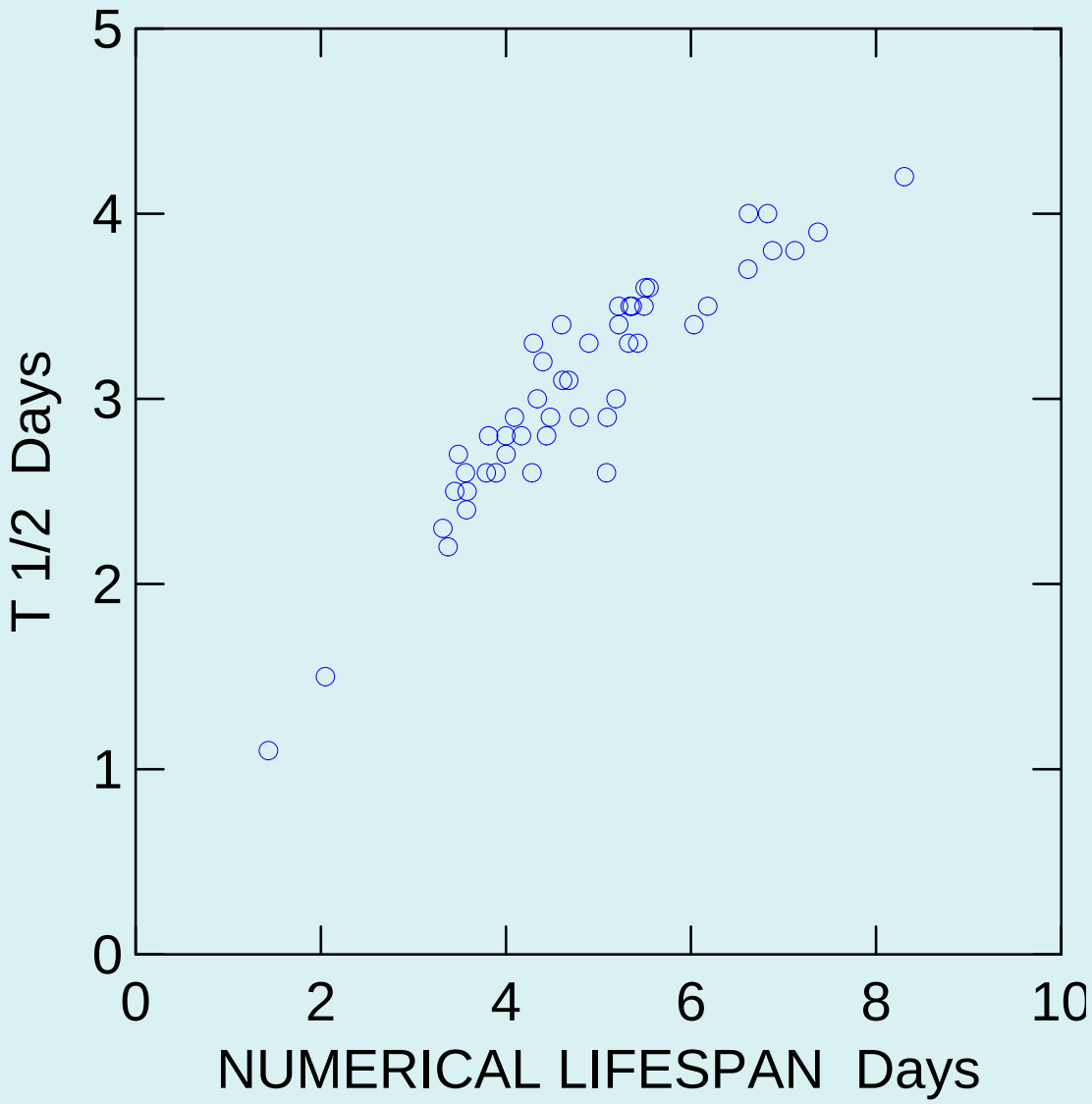

MEAN RESIDUAL LIFESPAN
Fresh platelets
% RECOVERY
70
60
50
40
30
20
10
0
0 1 2 3 4 5 6 7 8 9 10 11 12
DAYS
Mean Residual lifespan
= Area below surival curve / % Recovery
=5.5 Days

RESIDUAL vs. NUMERICAL EXPECTED LIFESPAN (WMF)
(5 vs. 7 day storage studies with RDPs in CLX bags)



T ½ vs. NUMERICAL EXPECTED LIFESPAN (WMF)

(5 vs. 7 day storage studies with RDPs in CLX bags)



SURVIVAL PARAMETERS: (5 vs 7 day storage studies with RDPs in CLX bags, Double label, n=24 pairs)

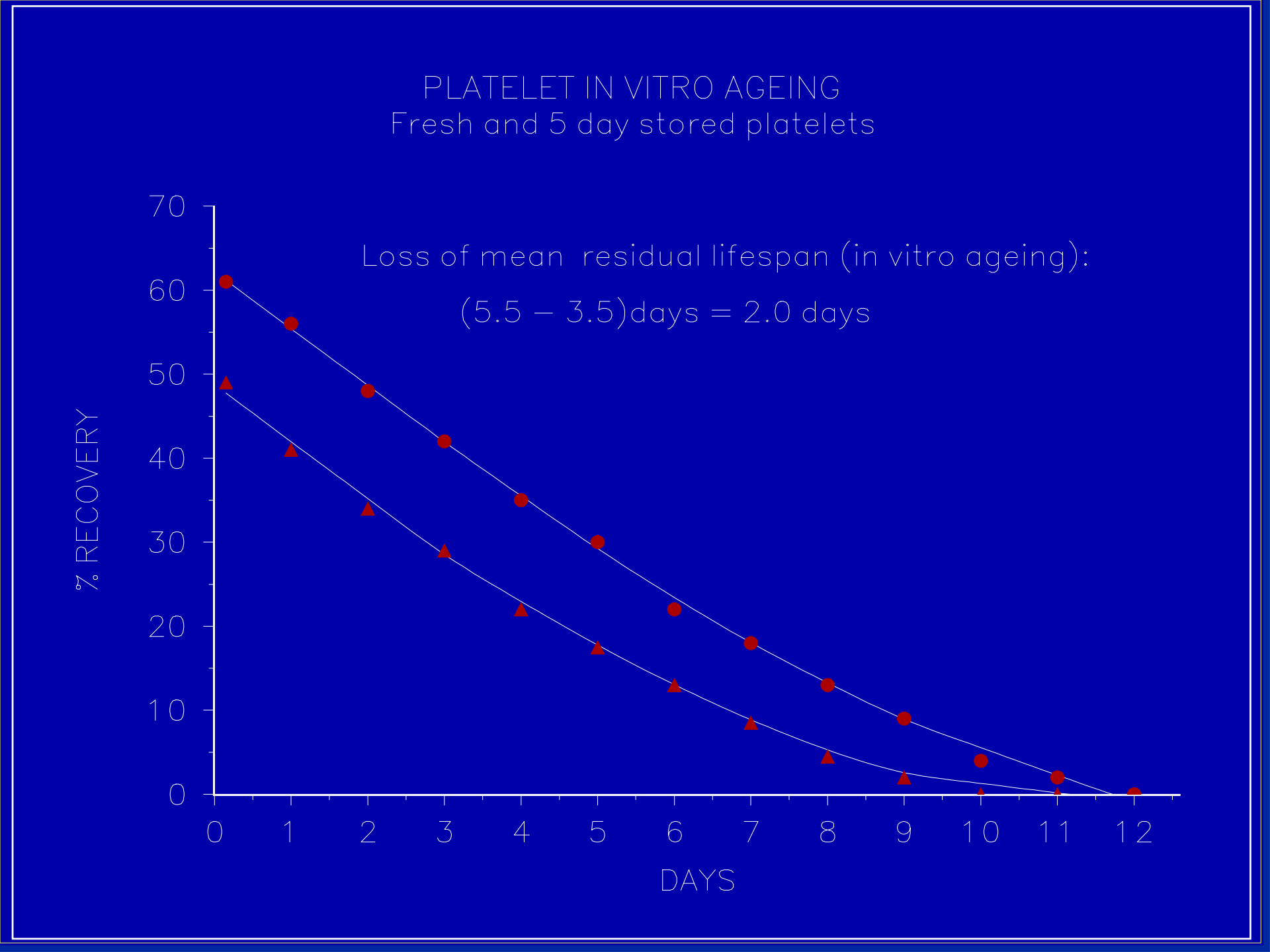
Parameter (by weighted mean function)	***Day 5 mean***	***Day 7 mean***	***Difference 95 % CI paired t-test***	***Probability paired t-test***
Numerical (Days)	5.3	4.4	0.5 – 1.4	<0.000
Residual (Days)	3.6	3.2	0.2 – 0.6	<0.000
T ½ (Days)	3.3	2.9	0.2 – 0.6	0.001
Random Destr. WMF (Exp. F.)	0.17	0.24	0.024- 0.12	0.005

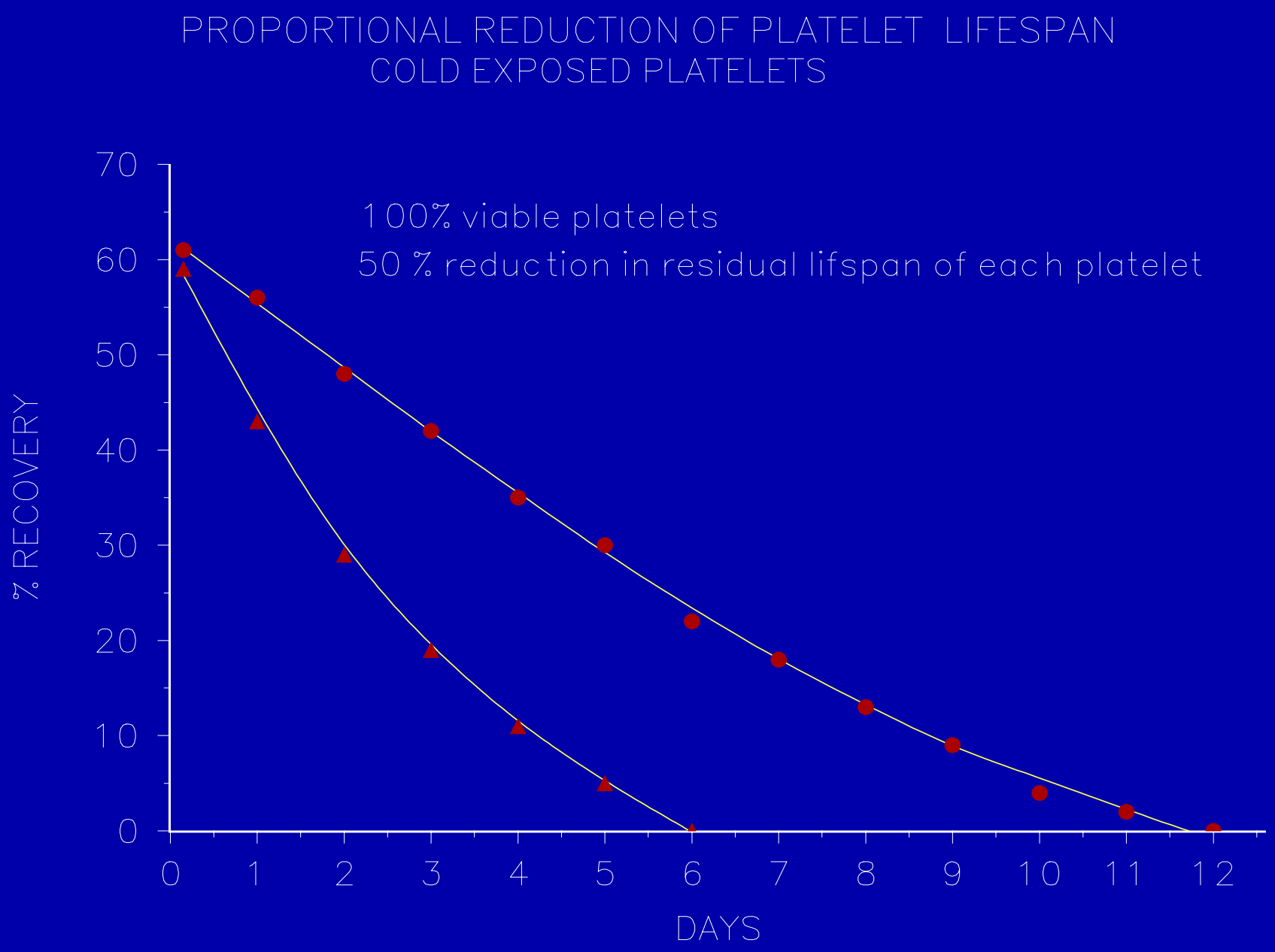
MATHEMATICAL MODELING

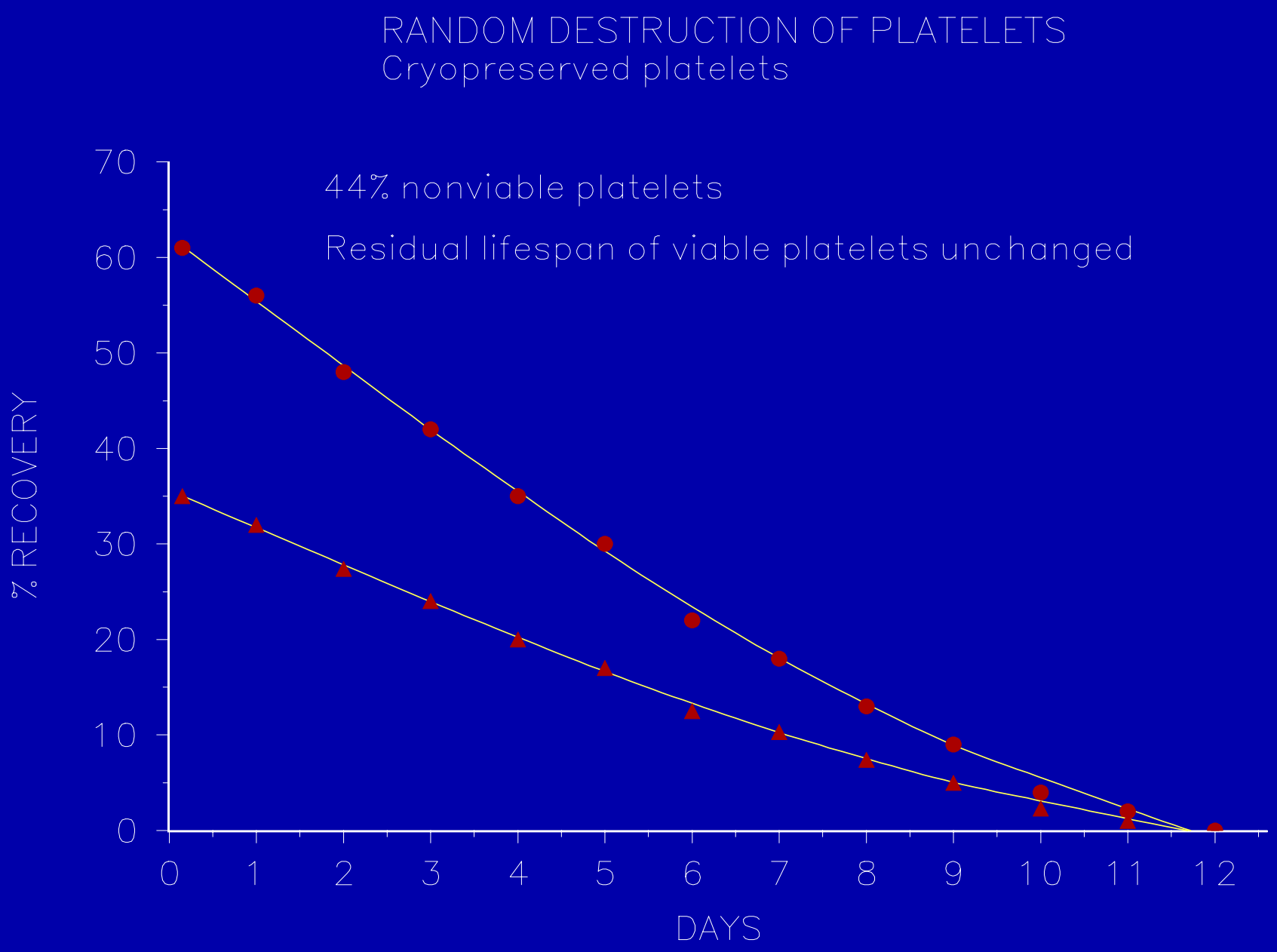
Comparison of the survival data of test platelets to that of fresh /control platelets may give useful information about the nature of a storage/process lesion.

Some parameters that can be calculated by appropriate mathematical models are:

- **Loss of % recovery due to aging versus due to random destruction**
- **Decrease in residual lifespan due to ageing versus random damage**


PLATELET IN VITRO AGEING
Fresh and 5 day stored platelets
Loss of mean residual lifespan (in vitro ageing):
(5.5 – 3.5)days = 2.0 days
% RECOVERY
70
60
50
40
30
20
10
0
0 1 2 3 4 5 6 7 8 9 10 11 12
DAYS


PROPORTIONAL REDUCTION OF PLATELET LIFESPAN
COLD EXPOSED PLATELETS
100% viable platelets
50 % reduction in residual lifspan of each platelet
% RECOVERY
70
60
50
40
30
20
10
0
0 1 2 3 4 5 6 7 8 9 10 11 12
DAYS


RANDOM DESTRUCTION OF PLATELETS
Cryopreserved platelets
44% nonviable platelets
Residual lifespan of viable platelets unchanged
% RECOVERY
70
60
50
40
30
20
10
0
0 1 2 3 4 5 6 7 8 9 10 11 12
DAYS

PROPOSED STEPS TO ENSURE ACCURACY OF VIABILITY MEASUREMENTS BY RADIO LABELING STUDIES

Variability Related To Donor

Inaccurate (and overestimated) Blood Volume based on current formulas for body surface area –

- **Better formula for calculation of blood volume**
- **Paired studies**

Labeling Method

Ensure uniform labeling of an representative population in a platelet product to be evaluated

- **Determine platelet loss during labeling (test vs. control)**
- **Determine platelet size distribution pre and post labeling**
- **Determine Isotope uptake/elution in various subpopulations (test vs. control product)**

PROPOSED STEPS TO ENSURE APPROPRIATE DATA ANALYSIS AND INTERPRETATION

Select data points to be included based on

- **Precision (more the better)**
- **Evenly spaced (clustered may cause biased results)**
- **Eliminate contribution of labeled RBCs**

Select appropriate mathematical models and parameters based on

- **Goodness of fit by residual sum of squares**
- **Robustness**
- **Informative about the nature of a potential lesion/improvement of a product**